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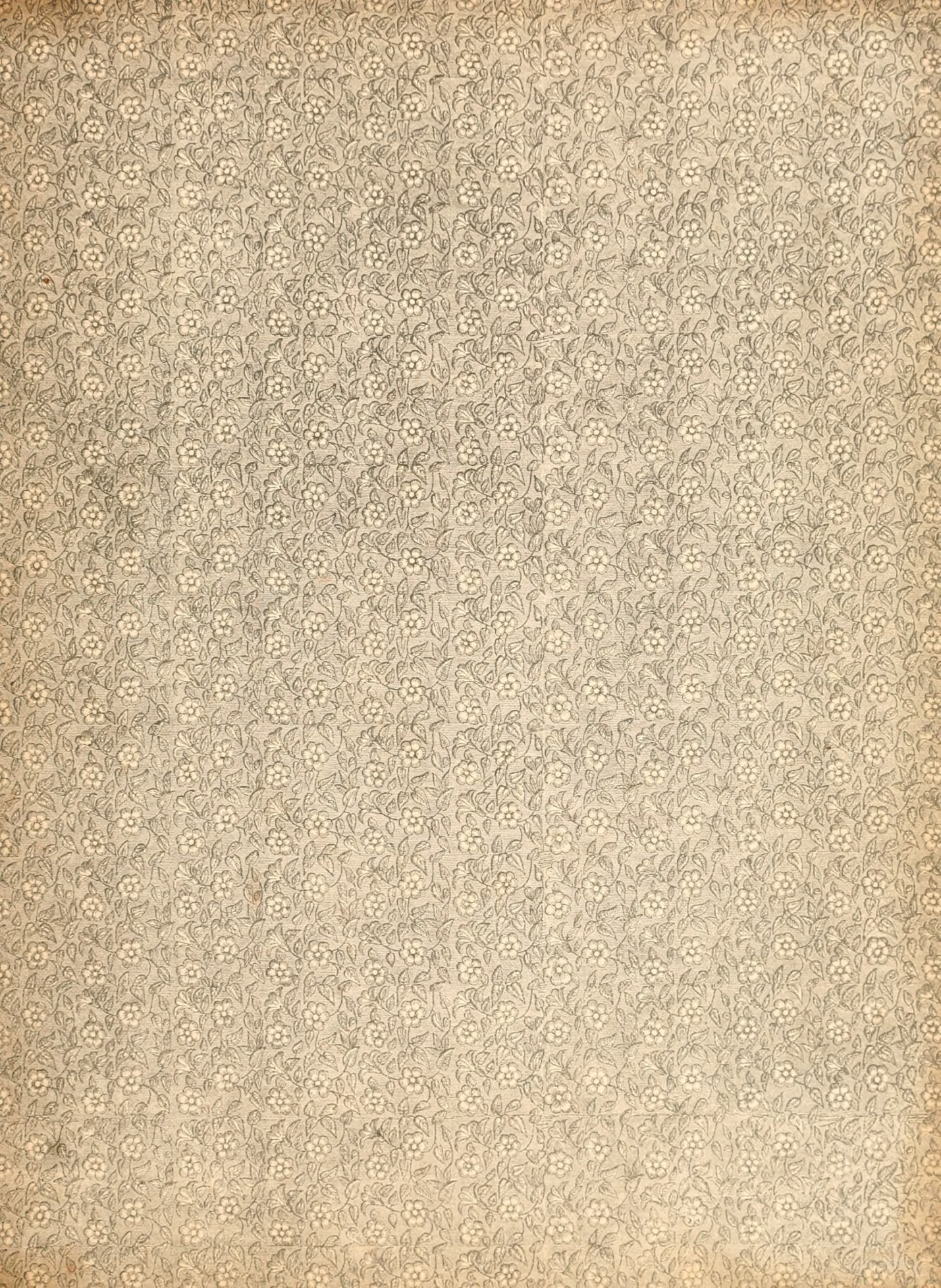


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SPEC. COLL.

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ON
SULPHONFLUORESCIN
AND SOME OF ITS DERIVATIVES.

DISSERTATION
Presented for the Degree
of
Doctor of Philosophy.
at the
Johns Hopkins University

by
C. Willard Hayes.

1917.

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On Sulphonfluorescein and some of its Derivatives.

Introduction.

The close analogy in composition and structure between phthalic acid and ortho-sulphobenzic acid suggests the possibility of obtaining from the latter, by its action on phenols, substances analogous to the phthalains. If such compounds could be made they would afford a favorable opportunity of studying the effects produced in the properties of a compound by the replacement of CO by SO₂. It was with a view to such study that the following described work was undertaken at the suggestion of Prof. Rinsen and carried on under his constant guidance.

Some experiments previously performed by Rinsen and Palmer, ^{et al.} indicated the

possibility of the formation of a fluorescent substance to the action of ortho-sulphobenzoic acid on noreine but this did not succeed in obtaining any definite crystalline compound from the reaction.

The chief obstacle to be overcome in the work is the difficulty in obtaining the o-sulphobenzoic acid - and a large proportion of the work here described was applied in that direction.

Ortho-sulphobenzoic acid: Methods for its preparation.

1. From toluene and H_2SO_4 .

A method employed by Ransen and Fahlberg (Am. Chem. Soc. 1881, p. 1) for putting the sulphonate acid group in the ortho position to methyl was; (a) treat toluene with fuming H_2SO_4 forming thus ortho- and para-toluene sulphonate acids, to make

the sodium salt of the sulphone and then
formed and from this the potassium salt.

(c) that this mixture of potassium toluene sulphonate
with phosphorus pentachloride forming the
corresponding sulphonochloride. One of these (para)
being a solid and the other (ortho) an oily liquid
a nearly complete separation could be effected.
One difficulty with this method is however
that the larger part of the product is the para
and only a comparatively very small pro-
portion of the ortho compound is formed.

2. From *p*-nitro-toluene and H_2SO_4 .

A second method employed consists in
starting with *p*-nitro-toluene. This when treated
with H_2SO_4 forms toluene *p*-nitro-sulphonic acid.
If now a method could be obtained for remov-
ing the nitro group the desired result would
be attained.

The attempt was made by Rosen and

Palmer, (47) to accomplish this by (a) reducing the nitro compound to the amino compound, the diazo compound and (c) boiling this with absolute alcohol. According to generally accepted views this should effect the removal of the diazo group and its replacement by hydrogen.

Experiments however showed that the replacement was made not by hydrogen but by the ethoxy group -OEt. This method was therefore impracticable.

A modification of this method was suggested by an observation of Baeys and Liebrmann that if phenyl hydrazine be boiled with a dilute solution of copper sulphate the hydrazine group is replaced by hydrogen and benzene thus formed. Hence it was believed that if the hydrazine compound should be made from the diazo compound mentioned above, the corresponding hydrocarbon, i.e. toluene o. sulphonic acid could be obtained. The results of experiments showed

that this afforded a practicable method of preparing toluene ortho-sulphonic acid.

After experimenting with various modifications of the method the following was found to be the best adapted to the purpose.

The potassium salt of toluene p-nitro-o-sulphonic acid - is easily obtained, as already stated, by heating p-nitro toluene on the water bath with three times its weight of fuming H_2SO_4 , neutralising with caustic alkali and the solution of calcium salt - thus obtained adding a slight excess of K_2CO_3 . On filtering from the precipitated CaCO_3 and evaporating slightly, the salt is obtained in long needle shaped crystals of a pale straw yellow color. This is $\text{C}_6\text{H}_4\left\{\begin{array}{l} \text{SO}_3\text{K} \\ \text{NO}_2 \end{array}\right.$ (p)

The reduction of the nitro group is best effected by means of tin and HCl , in the proportions - salt 8 parts - tin 6 parts and concentrated HCl 30 parts.

The amido acid forms a compound

with tin which crystallizes from the HCl together with stannous chloride. This compound may be broken up and the tin removed by continued boiling with water.

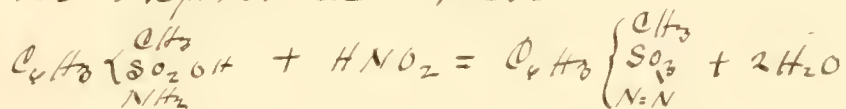
A better method of removing the tin is by dissolving the compound in Na_2CO_3 . This forms a salt with the amido acid and throws down the tin as SnCO_3 , a white flocculent precipitate. On filtering and adding to the solution conc. HCl, the free amido acid is deposited in characteristic colorless

rhombic crystals. Having the formula $\text{C}_6\text{H}_5 \begin{Bmatrix} \text{CH}_3 \\ \text{SO}_2\text{OH} \\ \text{NH}_2 \end{Bmatrix}$ (11)

The method at first employed for preparing the hydrogen compound consisted in treating the amido acid, suspended in HCl, with potassium nitrite and then with stannous chloride. The tin was then removed from the solution on the addition of sodium carbonate and the hydrogen compound thrown down with HCl. This method however gave

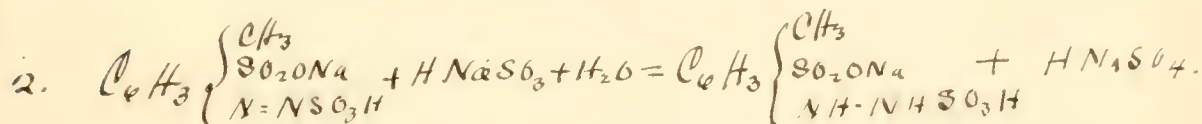
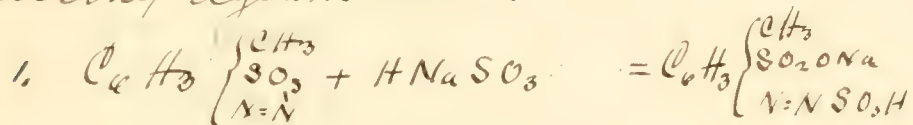
low yields the yield being only about 50% of the theoretical.

Another method was accordingly substituted for the above reaction, that of Staudenmaier and Bömer (Ber. N. 3 494). By this the diazo compound is made first and isolated. This is done by suspending the finely powdered acid in absolute alcohol, cooling and passing a current of the oxides of nitrogen through in the ordinary way. The acid changes in appearance becoming more crystalline and slightly darker and reacts quickly on being shaken. The reaction here may be expressed thus -



When the reaction is completed as shown by the appearance of the suspended powder it is filtered and while still warm is added to a solution of acid sodium sulphite as long as it continues to dissolve readily.

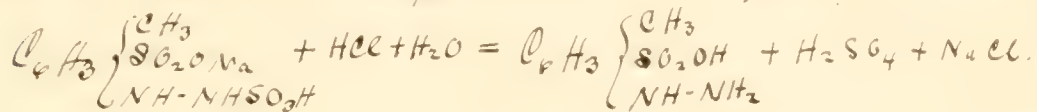
To this solution there is added a quantity of solution of acid sodium sulphite equivalent to that already used and the solution is then boiled. It has at first a deep red color but in a few moments becomes light reddish yellow. The reaction of HNaSO_3 on the diazo compound may be represented in two steps, the first portion forming an addition-product and the second acting as a reducing agent. Thus.



To the hot solution an excess of conc. HCl is added when the hydrazine compound appears in a few moments in lustrous yellow scales which completely fill the solution. On the addition of the HCl a large amount of SO_2 is given off from the scales of HNaSO_3 and

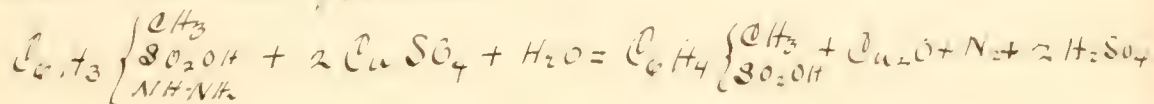
the solution becomes deeper. When the hydrazine has separated the mother liquor is again yellow.

The reaction is represented as follows:-



The yield of hydrazine when both the amino and the NaHSO_3 are freshly prepared is practically quantitative.

The hydrazine thus prepared was treated with a hot 10% solution of copper sulphate till a permanent blue color was obtained in the solution. Nitrogen is evolved and the copper sulphate is reduced to cuprous oxide which is precipitated as a red powder. The reaction is as follows.



Chalk was added to the solution to precipitate the H_2SO_4 and form a calcium salt of toluene-O-sulphonic acid. From this the sodium salt was made by adding a slight excess of Na_2CO_3 and

evaporating to dryness. The salt is very soluble being deliquescent in the air while its corresponding potassium salt is not. From 1538 gr. of para-nitro-toluene, 655 gr. of toluene ortho sodium sulphonate were obtained.

Having thus obtained the toluene-ortho-sulphonic acid the next step in the problem was to find a convenient method for converting this into ortho-sulphobenzonic acid. Two ways present themselves for accomplishing this end.

(1) direct oxidation of this salt, and (2) conversion into benzonic sulphoxide from which the acid may be obtained. Both of these methods were tried.

Oxidation of toluene-o-sodium sulphonate. $\text{C}_6\text{H}_4\{\text{CH}_3\}_{\text{SO}_2\text{ONa}}$

The sodium salt of toluene-o-sulphonic acid is oxidized to ortho-sulphobenzonic acid with considerable difficulty by KMnO_4 in neutral solution.

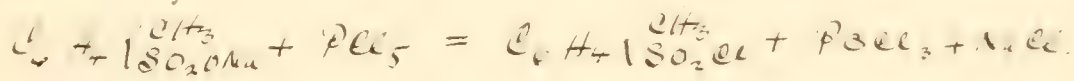
These two experiments showed that the oxidation was not complete after 24 hours boiling with excess of permanganate. If the solution be made alkaline, however, the oxidation is completed in a few hours, yet the greatest difficulty still remains in the separation of the free acid from the products of oxidation in the solution. If HCl be added to the solution the acid salt C_6H_4 ^{is formed} $(SO_2, OK)_2$ and this has nearly the same solubility as the HCl also present. A better method therefore is to add a slight excess of H_2SO_4 and evaporate nearly to dryness. In this way an ^{acid} sulphate and the free acid ^{are} ^{formed} ^{presumably}. The mixture is treated with alcohol (95%) which extracts the acid leaving the greater part of the inorganic salts. This extract is evaporated and reextracted with alcohol. To this solution $BaCO_3$ is added to precipitate the H_2SO_4 and form the Barium

salt of the *o*-sulphobenzic acid. The solution is filtered from the BaSO_4 and just enough H_2SO_4 is added to exactly precipitate the barium. The solution should then contain only the free acid sought which crystallizes out on evaporating to a small volume. While the method is theoretically possible it presents so many difficulties that it is practically useless. The yield is extremely small; only enough acid being obtained in this way to show that it was possible.

Formation of Sulphinide from toluene-*o*-sodium sulphonate.

The second method for obtaining free *o*-sulphobenzic acid from toluene-*ortho*-sulphonate acid is by the conversion of the latter first into benzic sulphinide and then into the free acid. The sulphinide was made essentially as described by Ransom (*Am. Ch. Soc. Vol. I. p. 428*) with a few changes in the details as follows.

The salt $C_6H_4(SO_2ONa)$ finely pulverized and in portions of from 10 to 50 gr. was placed in a Florence flask; an equivalent quantity of PCl_5 added; an inverted condenser was then attached and the flask shaken. The action takes place at once and requires sufficient heat to distil off the oxychloride formed in the reaction. This being returned to the flask by the condenser furnishes a liquid medium in which the reaction takes place more readily and completely than when it is not present. It is best to cool the flask at first and afterwards heat gently on the water bath. The reaction which takes place may be represented as follows.



On the addition of water the chlorine separates as a light yellow oil. This is washed with water and concentrated aqueous ammonia added which forms teluro-sulphuramide.



The reaction is accompanied by a slight evolution ^{of heat} and the formation, apparently, of an intermediate product having a yellowish color, which passes over on longer standing into the white amide. After standing several hours the excess of ammonia was driven off by very gentle heating on the water bath. If the heat is too high a large amount of a tarry product is formed and the yield of amide is correspondingly small. In any case some of this tarry product is formed. When nearly all the ammonia had been driven off the mass was boiled with water which dissolves everything except the tar. The hot solution was filtered through charcoal and on cooling the amide separates as white flaking crystals which melt at $155^{\circ}-56^{\circ}$.

The amide thus obtained was oxidized as described by Riesen (loc. cit.) with potassium.

permanganate in neutral solution. The proportions are 10 gr aniline, 40 gr KMnO_4 and 1% water. The oxidation was usually effected in from four to six hours.

To obtain the sulphinide from this solution after oxidation, the latter, after filtration from the precipitated oxides of manganese, was slightly acidified with HCl and evaporated to about one fourth its original volume. On the addition of concentrated HCl to this solution the sulphinide separated out in white or slightly yellowish feather shaped crystals melting at 212° and having the characteristic intensely sweet taste.

Formation of Sulphinide from Toluene by means of the chlorosulphonic acid reaction.

Before passing on to the method used for converting the sulphinide into formic acid another method should be described by which

the former was obtained in large quantities and much more easily than by the one above described.

Bicknells and Otto (Ber. 81. 2061) found that by treatment of toluene with sulphuryl hydrosulphuric or chlorosulphonic acid. $\text{Cl-SO}_2\text{OH}$, both α - and β - and as they suppose also meta-toluenesulphuric acid were formed together with the corresponding sulphonic acids.

Claesson and Wallin (Ber. 11. 548) repeated the work reaching practically the same results and finally Hayes (Am. Chem. Soc. Vol. VIII. p. 176) employed the reaction as a convenient method for obtaining toluene osulphuric acid.

Chlorosulphonic acid is made by passing dry HCl over solid sulphuric acid so long as it continues to be absorbed. Since no solid sulphuric acid was at hand ordinary fuming Nordhausen acid was taken and from one of two equal portions the SO_2 was driven

over into the other. HCl was passed into the latter and the resulting chlorosulphonic acid distilla off at about 156° .

This was placed in a flask, provided with a drop funnel and exit tube, in portions of 150 gr. and to each portion 60 gr of toluene was added, very slowly, with constant shaking, the temperature being kept near 10° .

The action is violent and if any toluene is allowed to collect on the surface of the liquid it is apt to produce disastrous results. Large quantities of HCl are given off and the liquid in the flask assumes a brown color. When all the toluene has been added, it is poured into a large quantity of ice water, when the sulphur chlorides separate out, the ortho as a heavy oil and the para as a white crystalline solid. After allowing to stand some time in order that as much of the para-chloride might crystallize as possible the ortho was

drawn off and subjected to a freezing temperature for several hours. By this means more of the *p*-chloride was removed and the operation was repeated as long as any crystals continued to form, generally two or three times. In this way the greater part of the para. may be removed, though some still remains dissolved in the liquid chloride, which cannot be removed by repeated freezing.

The chloride thus obtained was treated with strong aqueous ammonia. The conversion to the sulphamide does not take place so readily as ^{in case of} the pure *o*-chloride obtained from the sulphonic acid and phosphorus pentachloride.

After standing about two days the whole of the *o*-chloride had solidified to a yellowish brown mass. The excess of ammonia was driven off by gentle heating on the water bath and the mass then boiled with water. Not enough water was added at first for complete

solution but when the aq. solution was saturated it was poured off through a filter and from it the amine separated in yellowish feathery crystals which melt at $105^{\circ}-125^{\circ}$ and consist therefore as shown by Fehle (in Chem. Abstr. p. 170) of a mixture of o. & p. sulphonamides. It was recrystallized and from it was obtained a portion melting at $153^{\circ}-5^{\circ}$ and one at $108^{\circ}-20^{\circ}$.

Since this mixture could be completely separated by recrystallization another method was suggested. Ransom has shown that $\text{K}_2\text{Cr}_2\text{O}_7$ in acid solution does not oxidize the methyl group in $\text{C}_6\text{H}_4(\text{SO}_2\text{NH}_2)(\text{CH}_3)$ but does oxidize that in $\text{C}_6\text{H}_4(\text{SO}_2\text{NH}_2)(\text{H})$. It was thought that in a mixture of the two the former might be left unchanged while the latter was oxidized to p. sulphonaminobenzoic acid.

To test this 5 gr of the mixture, melting at $105^{\circ}-125^{\circ}$, was heated with 40 gr $\text{K}_2\text{Cr}_2\text{O}_7$, 55 gr H_2SO_4

and 2 vols. of water for about two hours. It was then tested and shown to be still a mixture of *o* & *p*. amides. Since it was again heated for several hours with half the original quantity of oxidizing mixture, then diluted, filtered and washed. The white crystalline residue was treated with sodium carbonate to dissolve the benzoic para sulphamide and the residue was found to be pure toluene *o*. sulphonamide melting at $53^{\circ}-55^{\circ}$. The small quantity remaining, 3 grs. indicated that part of the *o*. amide had been completely broken down by the strong oxidizing agent, though the proportion of *o*. & *p*. amides in the original mixture was known only approximately. The evolution of gas during the oxidation would point to the same conclusion.

Although this effects a complete separation it is hardly economical since it will be shown later that a separation can be con-

veniently effected after the oxidation with KMnO_4 so that the o. amide contained in the mixture need not be lost.

The original mass ^{was} treated with successive portions of water till nothing remained but a black tarry substance.

The amide which separated from these extracts was perfectly white and melted at 153.5° . It was therefore regarded as practically pure o. amide. The yield in amide melting above 153° was a little over one sixth the weight of toluene used.

The amide obtained in this way was oxidized in the manner already described. It was found however that there was always some benzoinic b. sulphonic in the solution after oxidation, due to the slight admixture of b. with the o. amide used. This is thrown down with the sulphonic on acidifying the solution and may be removed as a

crystallization since it is somewhat less soluble in hot and cold water than sulphinide.

A better way to effect the separation however was found to be the following. After many evaporations the solution containing the product of oxidation, neutralized with HCl to about one fifth its original volume it is made very slightly acid and allowed to cool. In this way very nearly all the benzoin β -sulphamide is separated from the solution and none of the sulphinide. After filtering, strong HCl is added and the sulphinide then separates in its characteristic form. This indicates that sulphinide forms an alkaline salt which is not decomposed by dilute HCl while the β -sulphamide does not.

The mixture of amides melting at 105° - 120° was oxidized and the products separated in this way gave about equal quantities of sulphinide and benzoin β -sulphamide.

When toluene is treated with chlorosulphonic acid there are formed besides the ortho and para chlorides also ortho and para sulphonics acids. These of course are in solution in the water from which the chlorides separate. In order to recover the ortho acid, the solution was neutralized with chalk forming the calcium salt: this converted into the potassium salt which by evaporating the solution to dryness was obtained as a white crystalline powder. When treated with PCl_5 in the manner already described this gave a mixture of ortho and para sulphonchlorides consisting of about $\frac{1}{3}$ ortho and $\frac{2}{3}$ para.

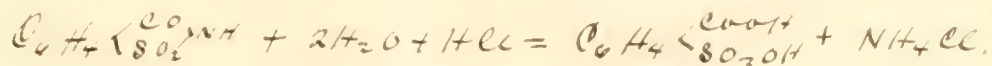
Formation of Orthosulphobenzene acid from Sulphinide.

Benzoin sulphinide may be converted into α -sulphobenzene acid by boiling with BaO and by treating in a closed tube with conc. HCl or by the evaporation on the water bath with HCl .

1. Three grammes of sulphinide were boiled in a flask connected with an inverted condenser for about two days with an excess of BaCl_2 . There was formed in the flask a hard mineral like mass which was insoluble in water and cold dilute HCl but dissolved in hot HCl with effervescence. This was a Barium salt, probably basic (?) of ortho sulphobenzoic acid. There was also formed an easily soluble barium salt of that acid. The former was dissolved in H_2SO_4 and treated with BaCO_3 ; the filtrate from the BaSO_4 which contained a soluble barium salt was added to that above mentioned and the barium exactly precipitated with H_2SO_4 and the filtrate evaporated to dryness giving the pure acid but not in a perfectly pure condition.

2. 2.75 gr. of sulphinide was sealed up in a tube with pure conc. HCl and ^{heated} two hours to 150° . On cooling nothing separated; the liquid

was evaporated to dryness giving 0.2 gr of acid and ammonium chloride. The reaction taking place here may be represented thus,



3. A more convenient method for obtaining the acid than either of the above, is to heat the sulphinic with conc. HCl on the water bath for two days. Then evaporate to dryness and dissolve the residue in a small quantity of water. If the sulphinic contained any persulphonic as is usually the case, this will be left undissolved and most of the NH_4Cl will crystallize on standing. This solution by slow evaporation deposits large colorless crystals of the free acid.

This acid is soluble in about two parts of cold water, very difficultly soluble in absolute alcohol and almost completely insoluble in ether. It does not melt under 250° but

considerably above that it melts, at first apparently without change and then with slight sublimation of a very deliquescent substance, probably the anhydride.

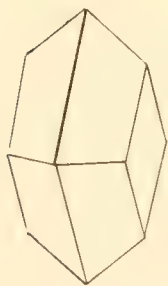
Two determinations of the S. made by Mr. A. F. Linn, gave the following results.

I .1358 gr substance gave .1555 gr BaSO_4 representing 15.72% S.

II

Calculated for the formula $\text{C}_6\text{H}_4\text{SO}_2\text{O}_2\text{H} = 15.84\% \text{ S.}$

A crystallographic examination of the acid showed it to belong to the orthorhombic system.



Axial ratios: $a:b:c = .880 : 1 : .8121$.

Planes. P and $\infty P \tilde{\infty}$.

Angles measured $\left\{ \begin{array}{l} P \wedge P \left\{ \begin{array}{l} \text{edge } x = 131^\circ 8' \\ \quad \quad \quad Y = 82^\circ 18' \\ \quad \quad \quad Z = 118^\circ 40' \end{array} \right. \\ P \wedge \infty P \tilde{\infty} = 114^\circ 38' \end{array} \right.$

The crystals are up to 3 mm. in length. The pyramidal faces are generally etched so that the image is poor.

Sulphonfluorescein.

Several attempts had already been made to obtain from the action of a sulphonating acid and rosein a substance analogous to the "fluorescein obtained by Bayer" from lithalic anhydride and rosein but which a strongly fluorescent substance was easily obtained no definite compound could be separated from it. Thus Palmer obtained by heating together the above named substances to 160° (?) a solid mass, part-soluble in water and part-insoluble as a dark brown amorphous powder. Both parts gave a strong fluorescence with alkalis. He was unable however to obtain the substance itself or any derivative in a crystalline form.

The first experiments in this series gave the same negative results. The mixture of acid and rosein was heated in a sulphuric

1) *Annalen*. No. 183. S. 1. No. 202. S. 36 & S. 153.

Beichte. No. IV. S. 457. 555. 658. 662.

" " VIII. S. 66. 146.

acid bath for several hours to 150° - 170° and as it showed no sign of solidification the temperature was raised to 200° and then to 235° where it was kept several hours longer. The black viscous mass obtained in this way became vitreous on cooling, and in all respects resembled that described by Palmer. This however is not the normal course of the reaction as shown later but is probably due to a decomposition of the normal product produced by too high heating.

An experiment was made with the acid salt of ortho-sulphobenzoyl acid. $C_6H_4(SO_3H)(COOH)$, urea and H_2SO_4 heating the mixture to 150° - 170° . A solid black mass was obtained strongly fluorescent in alkaline solution and in all other respects like the substance obtained above.

Preparation of Sulphonfluorescein.

As the result of a number of experiments the

following method of preparing and purifying the sulpho-fluorescein was found to give the best results.

The free acid is mixed with neoncin in the proportion of 1 part acid to 1.2 parts neoncin, or a slight excess over two molecules of the latter to one of the former. The mixture is placed in a deep vessel, a test tube or beaker, which is placed in a sulphuric acid bath, a thermometer being suspended in the mixture. The bath is heated and at about 100° the neoncin melts and the acid slowly dissolves in the liquid. No action appears to take place till the temperature reaches 125° when water begins to be given off and the liquid slowly assumes a salmon color. White crystals of neoncin collect on the sides of the vessel. After the heating has been continued for about seven hours at 178° - 185° the liquid has a clear deep red color but shows no

signs of becoming viscous. At length distinct yellow crystalline plates appear in the liquid and soon the whole mass becomes a thick nearly solid yellow paste. Continued heating at the same temperature causes no further apparent change. This mass which on cooling is made up of yellow crystals embedded in a red vitreous matrix, is then dissolved in hot water forming a clear red solution or at most one containing but a small quantity of a brown flocculent precipitate. This solution is filtered if necessary and evaporated to a small volume from which the substance separates on cooling in reddish yellow radial crystals.

These are filtered and washed with ether till the washings are perfectly colorless. On evaporation and repeated crystallization from water has a pale straw yellow color and when deposited slowly forms transparent crystal blocks from 2-6 mm

long, arranged in radial groups.

A considerable amount of mesoxcin is lost to sublimation during the reaction especially if the operation is carried on in a flask so that some excess should be added. But even when the mesoxcin is present in excess at the end of the reaction some free acid is always left which may be obtained from the mother liquors in the characteristic colorless orthorhombic crystals.

The water of crystallization and sublimum were determined in the new compound. In estimating the water, the substance was heated to 106° - 127° for about ten hours till it attained a constant weight. On standing in the air it quickly regains its original weight. When heated to 200° - 400° for some time it turns slightly reddish and loses some 10% of its weight which is not regained by standing in the air.

Estimation No. I. was made by fusion with KOH

and KNO_3 . Nos. II and III were made in Mr. Mindileff by Maxer's method, oxidizing with $KMnO_4$ in KOH solution.

.3882 gr. heated to $106^\circ-123^\circ$ lost .0302 gr. = 8.5 %

Calculated for $2H_2O - C_{19}H_{12}O_6S + 2H_2O -$ = 8.9 % water.

I .2007 gr sub. gave .286 gr $BaSO_4$ = 8.77 % S.

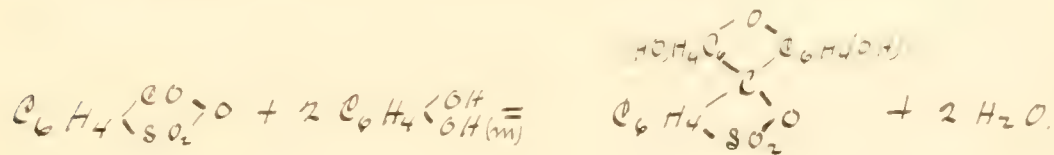
II gr " " gr " = 7.91 " "

III gr " " gr " = 7.85 " "

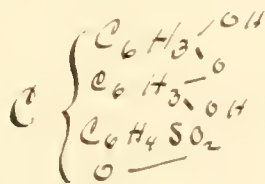
Calculated for $C_{19}H_{12}O_6S + 2H_2O$ = 7.92 % S

These analyses show with but little doubt that the substance has the composition indicated above i.e. $C_{19}H_{12}O_6S + 2H_2O$. The reaction therefore which takes place between ortho-sulpho benzoic acid and mesene from its analogy to that taking place between phthalic anhydride and mesene may be represented thus, as shown by Bayer in his second paper. (Ann 202. S. 43)

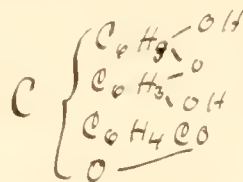
Representing the formation of the anhydride as the first action. $C_6H_4 \begin{smallmatrix} COOH \\ SO_2OH(O) \end{smallmatrix} = C_6H_4 \begin{smallmatrix} CO \\ SO_2 \end{smallmatrix} O + H_2O$ and the action of resorcin on this anhydride thus.



The substance thus formed would naturally receive the name Sulphonfluorescein from its analogy with Fluorescein.



Sulphonfluorescein



Fluorescein.

Properties of S. fluorescein.

This compound shows a marked similarity to the fluorescein described by Bayer as would naturally be expected from its great similarity of composition and constitution, but it also shows decided differences which may be attributed to the

replacement of Co by Sb_2 .

Because in water it shows a weak green fluorescence which in alkaline solution becomes much deeper but not by any means so strong as that of fluorescein. The dilute alkaline solution to transmitted light is almost perfectly colorless and by reflected light a clear green. Unlike fluorescein it is extremely soluble in water, about one part in two or three of hot and five or six of cold water. It is also soluble in absolute alcohol forming a yellow solution with weak fluorescence. It is soluble with difficulty in ether but when in solution is deposited only on evaporating to a small volume.

It does not melt at 250° but if held at a lower temperature for a long time becomes red undergoing some decomposition. If quickly heated somewhat above 300° it melts to a deep red liquid and then solidifies. If

the mass is treated with water it partly dissolves leaving a dark brown flocculent precipitate which dissolves on the addition of an alkali, the solution having an intense fluorescence nearly if not quite equalling that of fluorescein. This change, produced by heating was not further studied.

The crystals are very thin blades, apparently monoclinic, showing the Clinopneucoid $\infty P \infty$ and a very narrow prism ∞P and clinodome $P \infty$. The angle $\beta = 75^\circ$ and the extinction angle against the c axis = 20° . The axial ratio could not be accurately determined.

Salts of Sulphonfluorescein.

The influence of the SO_2 group is shown by the fact that the substance acts as an acid decomposing carbonates and forming salts which is not the case with fluorescein.

Barium salt.

The substance was boiled with an excess of carefully purified BaCO_3 for two hours. The filtrate from the BaCO_3 was evaporated to a small volume deposited yellow crystals resembling the original substance and in shape and texture. These were twice recrystallized and had then a light-straw yellow color.

A determination of the Ba. gave the following results. The salt was dried in the air.

I	.078 gr salt	gave .0304 gr BaSO_4	= 15.73 % Ba.
II	.1671 " "	.0757 " "	= 15.53 " "
III	.2425 " "	.0680 " "	= 15.65 " "
IV	.2866 " "	.0798 " "	= 15.57 " "
V	.1843 " "	.0498 " "	= 15.08 " "
VI	.2626 " "	.0708 " "	= 15.08 " "
VII	.3230 " "	.0906 " "	= 15.65 " "
VIII	.2875 " "	.0807 " "	= 15.66 " "

Calculated for $\text{C}_9\text{H}_{13}\text{O}_7\text{S}_2\text{Ba}$ = 15.10 % Ba

In the above determinations the salt analysed was taken from specimens made at three different times and purified in slightly different ways, Nos 1, 2, & 3 being washed with absolute alcohol. Nos IV and V were made by precipitating the Ba with H_2SO_4 from a solution of the salt.

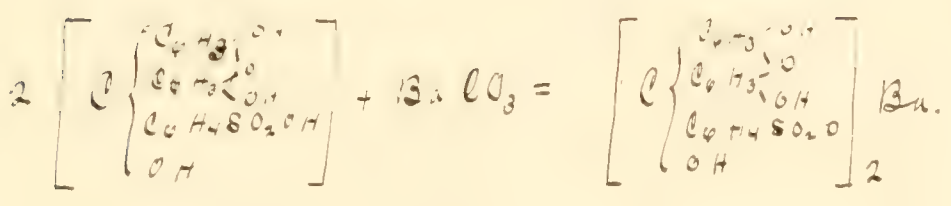
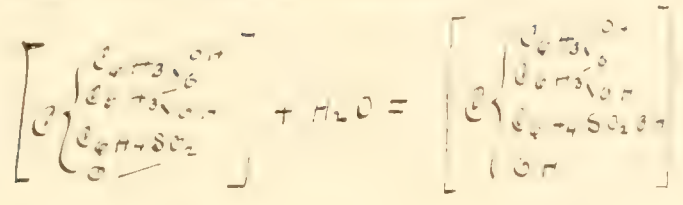
The water was determined by heating at 110° till constant weight was reached. Part only of the weight lost was ascribed to loss of water, in that

$$.0943 \text{ gr salt lost at } 110^\circ - .0286 \text{ gr} = 7.26\% \text{ } ^5_5$$

$$\text{Water calculated for } \text{C}_9\text{H}_{13}\text{O}_7\text{S Ba} + 2\text{H}_2\text{O} = 7.84\%$$

Although these analyses show a deficiency of Ba. somewhat above that required by a compound having the formula $\text{C}_9\text{H}_{13}\text{O}_7\text{S Ba}$, still this appears to be the most probable formula which can be assigned to the substance. If this is the true composition of the salt, then in sulphofluorescein the anhydride condition

was a mixture of the same with BaCO_3 formed in the solution.



By treating the salt with H_2SO_4 the original substance is reformed.

Calcium Salt.

Attempts were made to prepare the calcium salt but without success. The 8-fluorescein was boiled several hours with very finely powdered calcium carbonate and some salt was formed as shown by the CO_2 evolved but on evaporating the solution and reprecipitating the substance deposited it was found to be the unchanged 8-fluorescein. Some Ca. salt was in the mother liquors but its extreme solubility prevented a separation being made.

Acetyl derivative of S. fluorescein.

S. fluorescein was boiled with an excess of acetic anhydride for about three hours. The solution became quite viscous and when concentrated on the water bath left a black tar residue. This was treated with water which necessitated a fair amount of acid to dissolve the precipitate. The solution was boiled with animal charcoal and evaporated nearly to dryness. On cooling there separated a light yellow flocculent precipitate on which in hot water and but slightly less so in cold. This was dissolved in a small quantity of alcohol from which it separated on evaporation in small radial crystals having a light brown color, satiny luster. They also have a peculiar odor resembling slippery elm which is not removed by recrystallization. They show a tendency to become more

on exposure to the air. The substance does not melt or change in appearance under 245° . With alkalis it gives a slight greenish fluorescence. From the method of its formation this was taken to be an acetyl derivative of 8-fluorecein but whether the mono- or di-acetyl could not be determined without analysis for which the substance did not suffice.

Bromine substitution products of 8-fluorecein.

It was especially interesting to see what influence the SO_2 group would exert upon the introduction of Bromine into the compound. In the case of fluorecein some Bromine atoms enter easily and special precaution is necessary to obtain a product containing a smaller number. The case here is different with 8-fluorecein.

The latter was dissolved in glacial acetic acid in which it is soluble with some difficulty.

and to the solution was added a 20% solution of bromine in acetic acid, in sufficient quantity to make eight atoms of bromine to one molecule of 8-fluorescein. This solution was evaporated on the water bath and while still having a considerable volume, small red, simply rhombic crystals began to appear. The solution was evaporated to a small volume and allowed to cool but nothing further separated. These crystals are sufficiently soluble in water alcohol and ether. The alkaline solution shows a green fluorescence and slight red color by transmitted light. These crystals were dissolved in a large quantity of alcohol which on evaporation gradually deposited very small yellow crystals, which were dried in the air and taken for analysis. The Br. was determined by Carius method.

I. 2.345 gr sub. gave 1.718 gr AgBr = 31.17 % Br.

II. 2.786 gr " " 1.815 gr " = 27.72 % Br.

Calculated for $\text{C}_{19}\text{H}_{10}\text{Br}_2\text{O}_6\text{S}$ = 20.2% Br

These results though not conclusive indicate that under the given conditions it is the disform sulphonfluorescein which is formed. Whether this is due to the presence in the compound of the SO_2 group or simply to the greater insolubility of the di-form of the titration product cannot be definitely stated.

When the enormous active ocean mother liquor was evaporated to dryness, a red non-crystalline substance remained which more slowly resublimed eosin than the crystals. The concentrated alkaline solution had a deep red color without fluorescence and acted as a red dye stuff. The dilute alkaline solution showed the characteristic delicate pink of eosin.

Action of H_2SO_4 on S-fluorescein.

A test-tube in which S-fluorescein was being made just at the end of the reaction tube

and allowed the contents to pour out into the sulphuric acid bath, which had a temperature of 145° . On standing several days the solution deposited a heavy precipitate which was separated by filtering through glass wool.

When dry it formed a light yellow powder extremely soluble in water, alcohol and ether.

The alkaline solution had an intense green fluorescence with decided shade of pink by transmitted light. On account of its great solubility it was impossible to purify it by crystallization. Hence the Ba salt was made. The substance decomposed BaCO_3 with great ease forming an easily soluble salt. When it was attempted to evaporate the solution of this salt to crystallization the bath came out in a hard insoluble granular form and on continuing boiling of the solution turned brown. To avoid this undesirable form it was converted into the Ca salt by treatment

with H_2SO_4 and then $CaCO_3$. This also formed a hard granular insoluble mass on boiling but did not change in color. There was no guarantee as to its purity and only a small quantity was obtained it was not analyzed.

Action of HCl on S-fluorescein.

Hydrochloric acid does not dissolve S-fluorescein but converts it into a light-yellow granular mass. When recrystallized from water in which it is quite easily soluble it melts initially at 130° apparently with some decomposition. This compound was not further studied.

Reduction of S-fluorescein.

When treated with zinc dust in a strong alkaline solution sulpho-fluorescein is reduced to a colorless substance broadly analogous

to fluorescein which is formed in the same manner. On account of its great solubility it could not be obtained in the free state. It is quickly oxidized to o-fluorescein by oxidizing agents as KMnO_4 and HNO_3 and is also oxidized spontaneously on standing in the air. The latter action is however much slower than in case of fluorescein.

Conclusion.

The principal results relating to o-fluorescein which have been reached in this work may be briefly summarized as follows. Orthosulphobenzic acid acts on resorcin at a ^{temperature} of about 180° giving off water and forming a substance analogous to fluorescein but having the CO group replaced by SO_2 . This substance sulphonfluorescein crystallizes from water in light-yellow monoclinic crystals having the composition $\text{C}_{19}\text{H}_{12}\text{O}_6\text{S} + 2\text{H}_2\text{O}$. It is very soluble in alcohol and water and with difficulty in ether. It does not melt under 250° but above 300 melts with

decomposition. It shows in alkaline solution a clear green fluorescence. It acts as an acid, decomposing carbonates and forming salts, the 3rd salt having the composition $C_{19}H_{13}O_7S_2Na$. It forms an acetyl compound when boiled with acetic anhydride. It forms substitution products with Br, probably the dibrom-product most easily. It forms a compound with H_2SO_4 probably a substitution product, whose composition was not determined. It is reduced in fine dust and KOH to a colorless substance analogous to fluorescein.

Finally in terms of the present theory the substance itself may be represented thus -

